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TITLE: The Role of "Backup" NHEJ Repair in Creating Genomic Instability in CML

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14. ABSTRACT <ul style="list-style-type: none"> The BCR-ABL1 fusion gene in Philadelphia (Ph)-+ve chronic myeloid leukemis (CML) encodes a constitutively active tyrosine kinase that causes uncontrolled cellular proliferation. BCR-ABL1 expression results in elevated levels of reactive oxygen species (ROS), an increased incidence of DNA double strand breaks (DSBs), error-prone repair and genomic instability. We recently demonstrated that an error-prone alternative (alt) NHEJ pathway involving DNA ligase IIIa/XRCC1 is upregulated in CML cells. "Knockdown" of alt NHEJ proteins causes decreased DNA repair and an increased frequency of DSBs, indicating that this pathway is important for the survival of BCR-ABL1 positive cells, and so may be a specific therapeutic target (Blood publication, 2008). In the one year extension of the grant, we have shown that BCR-ABL modulates expression of alt NHEJ proteins. As an additional task, we have also examined the effect of recently identified alt NHEJ inhibitors in CML cells and show that they significantly decrease the survival of imatinib resistant BCR-ABL1+ve cells, compared with BCR-ABL1 cells that are imatinib sensitive and normal cells. These results suggest that alt NHEJ proteins may be therapeutic targets in CML that are sensitive to imatinib. 					
15. SUBJECT TERMS Main non homologous end-joining repair (NHEJ) , Back-up or alternative NHEJ repair, double strand breaks (DSBs), chronic myeloid leukemia (CML), BCR-ABL protein P210, DNA ligase inhibitors, Poly-ADP ribose transferase PARP-1 inhibitor.					
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Introduction

Background:

We and others have recently shown that the oncogenic BCR-ABL fusion protein in Philadelphia (Ph)-positive CML initiates a cycle of genomic instability that results in the acquisition of further genomic changes that can contribute to disease progression¹. BCR-ABL produces increased reactive oxygen species (ROS) that leads to DNA damage, including double strand breaks (DSB)². In turn, key facets of genomic instability in cancer and leukemia are caused by alterations in the pathways that repair DSB. We have demonstrated significantly increased error-prone repair of DSB in CML cells mediated by the non homologous end-joining (NHEJ) pathway, one of the main pathways for DSB repair in mammalian cells^{3,4}. However, the mechanisms biasing for this increased error-prone repair component of NHEJ in BCR-ABL-positive CML has yet to be clarified.

Purpose of research:

This proposal seeks to build on our preliminary data that provides a mechanism for the reduced repair fidelity in BCR-ABL-positive CML. In this scenario, double strand breaks (DSBs) produced by increased reactive oxygen species (ROS) in BCR-ABL-positive chronic myeloid leukemia (CML) need to be processed before proper repair can occur. However, we find that one key protein responsible for this processing, Artemis, is down-regulated in BCR-ABL-positive CML. Concomitantly, we find upregulation of the proteins DNA ligase III α and Werners which are part of a novel complex of proteins, some of which are known to be involved in a minor “back-up” non homologous end-joining (NHEJ) or alternative NHEJ (alt NHEJ) repair pathway. We proposed that this **novel protein complex** processes and repairs ROS-induced DSBs thus playing a role in the survival of CML cells. However, the repair of DSBs is highly error-prone, thus contributing to the acquisition of genomic alteration that drive CML disease progression.

This proposal seeks to determine the role of the altered repair proteins in CML cells:

- ❖ **Task 1** (Specific Aim 1) To determine whether the DNA Ligase III/XRCC1/PARP WRN complex is recruited to in vivo DSBs.
- ❖ **Task 2** (Specific Aim 2) To determine that the fidelity of DSB repair can be altered by modulation of components of the DNA Ligase III/XRCC1/PARP/WRN protein complex and/or Artemis.
- ❖ **Task 3** (Specific Aim 3) To define the relationship between BCR-ABL and the protein complex containing DNA Ligase III, XRCC1, PARP and WRN we will modulate BCR-ABL expression in CML cells and test for formation of the “back-up” complex and repair infidelity.

Summary of research in the last year

In the one year extension of the grant:

- ❖ We have completed Task 3, showing that BCR-ABL modulates expression of alt NHEJ protein DNA ligase III α . This regulation of alt NHEJ could occur directly or indirectly.
- ❖ As an additional task not written in the original proposal, we have used inhibitors of alt NHEJ proteins in CML cells to determine their role in cell survival. We show here that these DNA repair inhibitors significantly decrease the survival of imatinib resistant BCR-ABL1+ve cells, compared with BCR-ABL1 cells that are imatinib sensitive and normal cells. These results suggest that alt NHEJ proteins may be therapeutic targets in CML that are resistant to imatinib.

Summary of research for the entire granting period

- ◆ The BCR-ABL1 fusion gene in Philadelphia (Ph)-+ve chronic myeloid leukemias (CML) encodes a constitutively active tyrosine kinase that causes uncontrolled cellular proliferation. BCR-ABL1 expression results in elevated levels of reactive oxygen species (ROS), an increased incidence of DNA double strand breaks (DSBs), error-prone repair and genomic instability.
- ◆ We recently demonstrated that an error-prone alternative (alt) NHEJ pathway involving DNA ligase IIIa/XRCC1 is upregulated in CML cells. “Knockdown” of alt NHEJ proteins causes decreased DNA repair and an increased frequency of DSBs, indicating that this pathway is important for the survival of BCR-ABL1 positive cells, and so may be a specific therapeutic target (Blood publication, 2008).
- ◆ To determine whether alt NHEJ proteins that are upregulated in CML are important in cell survival, we used recently identified DNA inhibitors that were shown specifically to potentiate the cytotoxic effects of DNA damaging agents in cancer cells. Because PARP-1 inhibitors have been shown to specifically kill cells with defects in DSB repair, and BCR-ABL1-+ve cells have abnormal DSB repair, we have examined the effect of PARP-1 inhibitors on BCR-ABL1-+ve cells in the presence or absence of DNA ligase inhibitors. In preliminary studies, we have demonstrated that combining PARP and ligase significantly decreased the survival of imatinib resistant (IR) BCR-ABL1-+ve cells, compared with BCR-ABL1 cells that are imatinib sensitive and normal cells.

Potential impact of this study:

We have shown that upregulated alt NHEJ proteins in CML are important in cell survival. Inhibitors of alt NHEJ proteins could represent new therapeutic targets in CML cells that become resistant to standard therapy with tyrosine kinase inhibitors (TKI). This work could lay the groundwork for preclinical studies to determine therapeutic efficacy in patients with TKI-resistance.

Summary of progress

Completed Tasks in Previous Report

Task 1

(Specific Aim 1) To determine whether the DNA Ligase III/XRCC1/PARP/WRN complex is recruited to in vivo DSB.) We showed that in CML cells, proteins in the upregulated “back-up” repair complex, such as DNA ligase III and WRN localize to DSB following their induction.

Task 2

Specific Aim 2) To determine that the fidelity of DSB repair can be altered by modulation of components of the DNA Ligase III/XRCC1/PARP/WRN protein complex and/or Artemis.) We showed that siRNA down-regulation of these “back-up” repair proteins alter repair efficiency and fidelity. Importantly, we have determined that siRNA knockdown of DNA ligase III and WRN both cause an increase in the frequency of unrepaired DSB.

Task 3

Specific Aim 3) To define the relationship between BCR-ABL and the protein complex containing DNA Ligase III, XRCC1, PARP and WRN we will modulate BCR-ABL expression in CML cells and test for formation of the “back-up” complex and repair infidelity.

We showed that BCR-ABL stably expressed in CML cells leads to increased expression of DNA ligase III and WRN, members of an alt NHEJ protein complex.

Progress in the final year

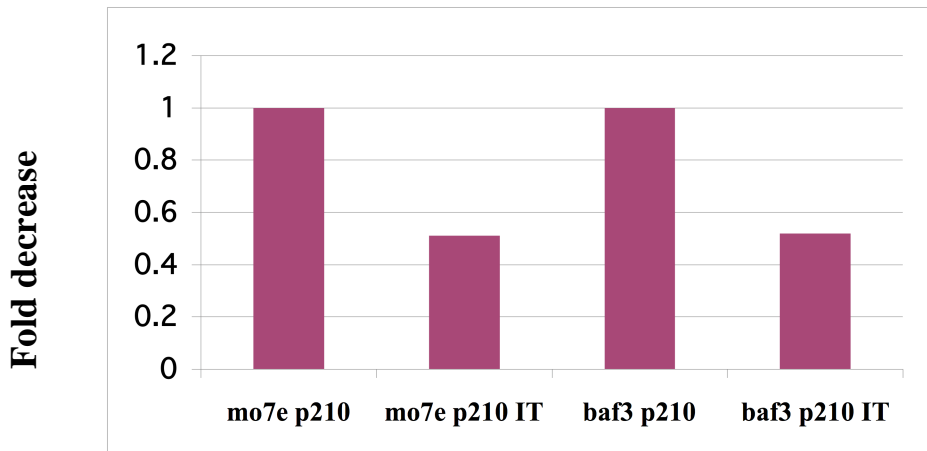
Completion of Task 3

Specific Aim 3) To define the relationship between BCR-ABL and the protein complex containing DNA Ligase III, XRCC1, PARP and WRN we will modulate BCR-ABL expression in CML cells and test for formation of the “back-up” complex and repair infidelity.

To determine if BCR-ABL protein expression is necessary for formation of the protein complex containing DNA Ligase III, XRCC1, PARP and WRN, we have abrogated expression of BCR-ABL by treating BCR-ABL cell lines (Baf3P210 and Mo7eP210) with imatinib (1 μ M for 72hrs) ⁵. Following imatinib treatment, we examined levels of “back-up” proteins DNA ligase III by Western analysis and found a decrease in expression compared with loading controls (**Fig. 1A**). Interestingly, we find that concomitant with the decreased levels of alt NHEJ protein DNA ligase III, classic pathway protein DNA ligase IV increases in expression (**Fig. 1B**). These data suggest that BCR-ABL may regulate the transcription of these DNA repair proteins. We also examined NHEJ repair in Baf3P210 cells following imatinib treatment, as we have previously described ⁶. Following imatinib treatment, the efficiency of repair increases, leading to a decrease in the frequency of repair errors. This suggests imatinib treatment may lead to a correction of DSB repair (**Fig. 2**).

A

Ligase 3

**B**

Ligase 4

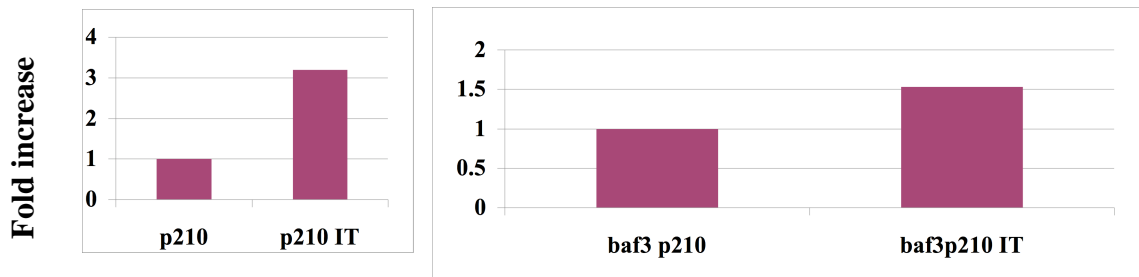
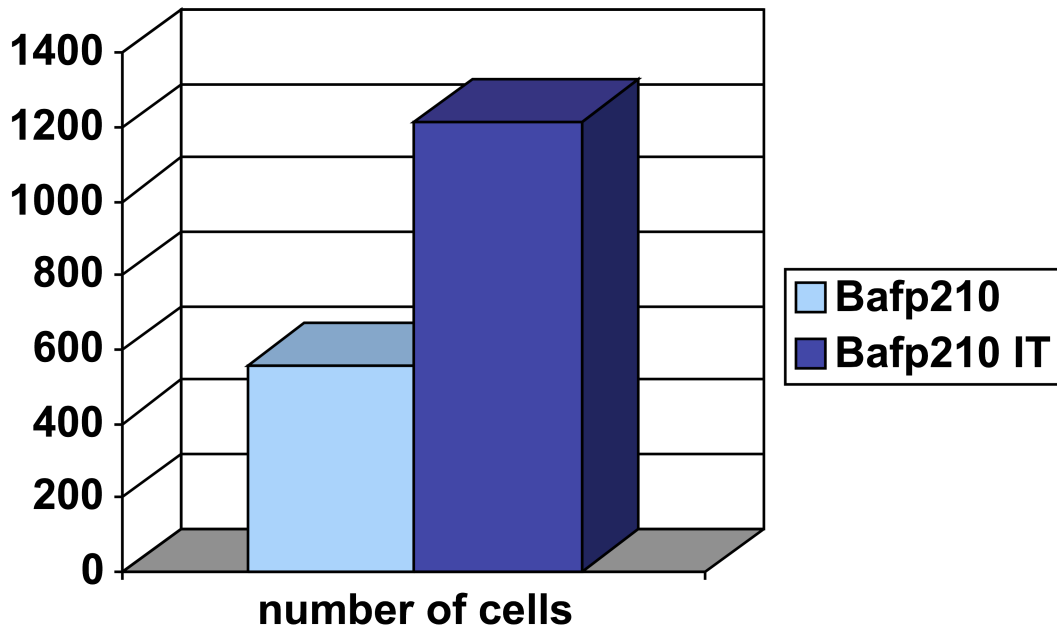


Figure 1 A: Bar graph showing relative decrease in ligase 3 after imatinib treatment (IT) for 48 hours compared to untreated cells. Western blotting in the cell lines Mo7e and Baf3 stably expressing the BCR-ABL fusion gene P210, for DNA ligase 3 using whole cell extracts. B-actin was used as a loading control. Extracts were prepared at 0 and 48 hour time points after imatinib treatment. **B:** Bar graph showing relative increases in ligase 4 after imatinib treatment for 48 hours compared to untreated cells. Immunoprecipitation in the cell lines Mo7e and Baf3 with XRCC4 following blotting with Ligase 4 using whole cell extracts. IgG was used as a loading control for the Mo7e cell lines whereas 5% of the extracts were used for western blotting against b-actin for the baf cell lines. Extracts were prepared at 0 and 48 hour time points after imatinib treatment

A] Repair efficiency



B] Misrepair frequency

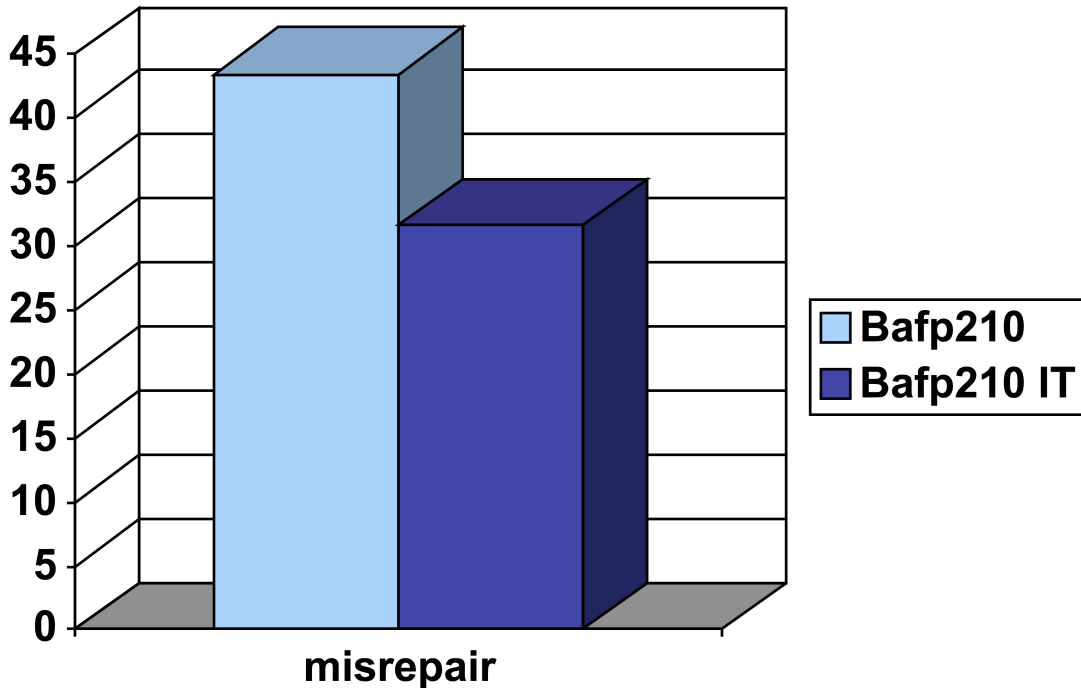


Fig. 2 An in vivo LacZ alpha plasmid reactivation assay was used to measure end joining in cells treated with imatinib for 48 hours. A] The total repair efficiency of the cells was measured. B] the misrepair frequency or repair errors was calculated as a percentage of the efficiency.

Additional Task

To determine whether inhibitors of alt NHEJ proteins can decrease survival of CML cells

Our published studies have shown that expression of the BCR-ABL1 tyrosine kinase initiates a cycle of genomic instability that is likely to promote disease progression. Specifically we have shown that expression of BCR-ABL1 induces production of ROS² and that cells transformed by BCR-ABL1, primary CML cells and cell lines established from CML patients have increased endogenous DNA double strand breaks (DSBs), as measured by immunostaining for γ H2AX foci^{4,7}. Furthermore, the repair of DSBs by NHEJ is abnormal and error-prone in CML cells^{3,7}. Recently, we have shown that the steady state levels of two components of the major DNA PK-dependent NHEJ pathway⁸, Artemis and DNA ligase IV, are significantly reduced in CML cells. Notably, there is a concomitant increase in the steady state levels of two components, WRN and DNA ligase III α ³, of a back up or alt NHEJ pathway that is characterized by microhomologies at the repair site, large deletions and translocations^{3,9}. Since we have shown that “knockdown” of ligase IIIa leads to decreased DNA repair and an increased frequency of DSBs in CML cells, this indicates that the alt NHEJ pathway is particularly important for the survival of CML cells, and so is an attractive therapeutic target¹⁰.

Using computer-aided drug design (CADD), Dr Tomkinson recently identified small molecule inhibitors that exhibit different specificities for the three human DNA ligases and are also active in cell culture assays. For example, L67 inhibits DNA ligases I and IIIa and L189 inhibits DNA ligases I, III, and IV. Concordant with their ability to inhibit DNA ligases in vitro, L67 and L189 are cytotoxic. Interestingly, at subtoxic concentrations, these inhibitors specifically sensitized cancer cells to DNA damage¹¹. Since CML cells overexpress DNA ligase IIIa, we chose to test DNA ligase inhibitor L67 that specifically inhibits DNA ligase I and IIIa as a potential therapeutic target. This drug was found to be cytotoxic at concentrations of >10mM, but at a subtoxic concentration of 0.3mM, L67 had a minimal effect on both BCR-ABL1 positive cells and controls (not shown). Since, PARP inhibitors have been used successfully in the treatment of cancers with DSB repair defects¹², we therefore examined the effect of L67 (0.3mM) in the presence or absence of the PARP-1 inhibitor Nu1025 (Calbiochem 50mM) in Mo7e cells stably expressing BCR-ABL1 protein (P210Mo7e), imatinib resistant P210Mo7e cells (P210Mo7eIR) and parental controls (Mo7e). P210Mo7eIR cells demonstrated a striking sensitivity to the combination of inhibitors, compared with the other cell lines (**Fig. 3**).

Since approximately 50% of IR in CML is conferred through mutations in BCR-ABL1 that affect the binding of imatinib to the onco-protein¹³, we next determined whether BCR-ABL1 was mutated. DNA sequencing of BCR-ABL1 revealed no mutations in P210Mo7eIR cells (not shown). Furthermore, BCR-ABL1 expression analysis of mRNA by qPCR and Western blotting analysis of proteins showed no change in steady state levels of BCR-ABL1 (not shown). However, examination of downstream targets proteins, such as, CRKL, ERK and AKT, by Western blotting analysis of IR and control cells exposed to imatinib, revealed that AKT is differentially phosphorylated in IR cells (**Fig. 4**).

To determine whether IR conferred by known mutations in the BCR-ABL1 gene are affected by the DNA ligase and PARP-1 drug combination, we examined two BCR-ABL1 mutations, E255K and T315I that are frequently detected in the cells of IR CML patients. Baf3 cells stably expressing E255K show an approximately 50% decrease in colony numbers, whereas those expressing T315I are unaffected by the combination of DNA ligase and PARP-1 inhibitors (**Fig. 5**). We have also compared the effects of this drug combination on bone marrow mononuclear cell (BM MNC) from TKI-resistant CML patients (N=4) with normal BM MNC controls. Two out of the four samples from TKI-resistant patients were sensitive to the combination of DNA ligase and PARP-1 inhibitors (**Fig. 6**). Interestingly, there were no detectable BCR-ABL1 mutations in sample #2, which exhibited the greatest sensitivity to the inhibitor combination. These data suggest that one subset of TKI-resistant patients that may benefit from this treatment strategy are those with no BCR-ABL1 mutations. The differential sensitivity conferred by different BCR-ABL1 mutations suggest that this treatment strategy may also be effective for a subset of these TKI-resistant patients with specific BCR-ABL mutations.

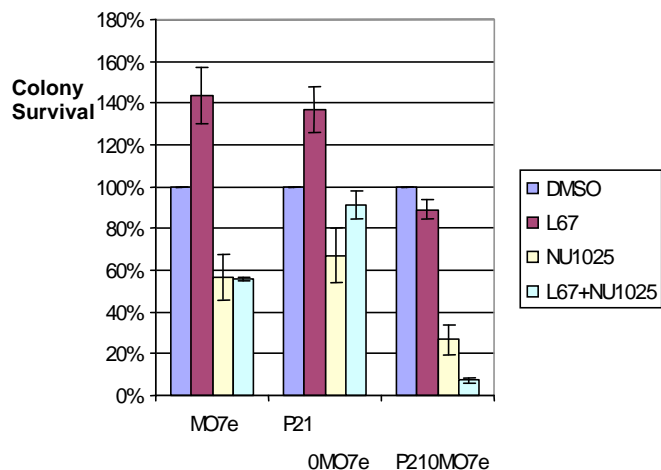


Fig 3. Colony survival of MO7e, P210 MO7e and imatinib resistant P210 MO7e IR cells after a 10 day growth in the presence of L67 (0.3uM) and NU1025 (50uM).

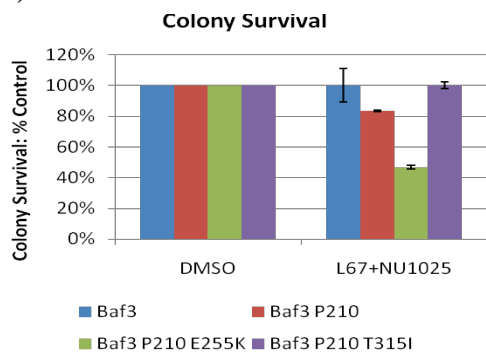


Fig 5. Colony survival of Baf3 cells stably expressing P210, and P210 mutants E255K and T315I following 10 day growth in the presence of L67 (0.3uM) and NU1025 (50uM)

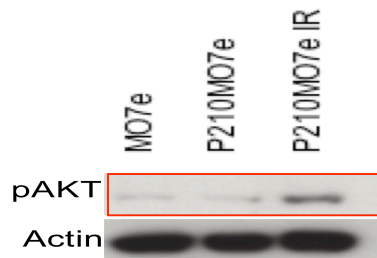


Fig 4. Western Blotting analysis of pAKT from proteins of MO7e, P210MO7e and imatinib resistant P210MO7eIR cells exposed to imatinib (1mM).

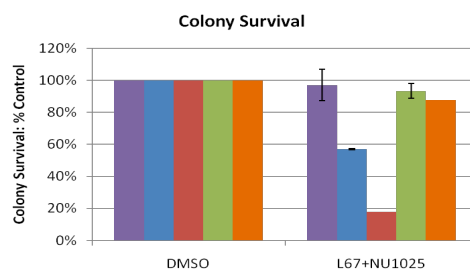


Fig 6. Colony survival of normal bone marrow (BCR-ABL negative; purple) and four patient samples after a 10 day growth in the presence of L67 (0.3uM) and NU1025 (50uM)

Key Research Accomplishments:

We report that:

- ❖ The **novel “Back-up” repair protein complex**, involving WRN and DNA ligase IIIa, we have identified in CML cells, **localizes to DSBs**.
- ❖ These proteins repair DSBs because their “down-regulation” (a) increases the number of **unrepaired DSB**, and (b) affects the **repair efficiency** in CML cells.
- ❖ Over expression of Artemis, the main NHEJ protein found to be down regulated in CML cells, increases correct repair of DSB.
- ❖ Upregulation of “Back-up” repair proteins, such as, DNA ligase III α is dependent on expression of BCR-ABL because stable expression of BCR-ABL in cells leads to increased expression of alt NHEJ proteins. Moreover, abolition of BCR-ABL expression in CML cells with imatinib leads to a decrease in DNA ligase IIIa and a concomitant increase in DNA ligase IV, a component of the main NHEJ pathway.
- ❖ We have demonstrated that inhibition of ligase in combination with a PARP inhibitor significantly decreased the survival of imatinib resistant (IR) BCR-ABL1-+ve cells, compared with BCR-ABL1 cells that are imatinib sensitive and normal cells.

Reportable outcomes:

- ❖ The aims of all tasks have been completed.
- ❖ We have a manuscript published in a high impact sub-specialty journal, Blood, describing this work¹⁰. (appendix).
- ❖ Based on this work we were also asked to write a review, which is now published¹⁴.
- ❖ We are preparing a manuscript reporting the effects of DNA repair inhibitors in CML cells.
- ❖ This work has resulted in presentation posters at the American Association of Cancer Research (AACR) and at the American Society of Hematology (ASH; appendix).
- ❖ This work has been presented in an invited talk at an international meeting in Bangkok, Thailand (appendix).

Conclusions:

- ◆ WRN and DNA ligase III α co-localize with DRneo at ISce-1 induced DSBs.
- ❖ siRNA down-regulation of WRN and DNA ligase III α results in increased DSBs and a decreased efficiency of repair.
- ❖ BCR-ABL expression leads to upregulation of DNA ligase III α and WRN.
- ❖ “Back-up” NHEJ or alt NHEJ proteins are involved in repair of excess DSB in CML cells and linked to CML cell survival.
- ❖ DNA ligase inhibitors in combination with PARP inhibitors lead to decreased survival of CML cells that are resistant to imatinib.
- ❖ Our results suggest that this aberrant protein complex is important in survival of CML cells that are resistant to standard therapy-tyrosine kinase inhibitors (TKIs).
- ❖ Thus inhibition of alt NHEJ proteins could be a novel therapy in CML patients who have failed therapy with TKIs and could lead to increased survival.

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Appendices

1. Published Abstracts

American Association of Cancer Research April 12-16, San Diego 2008

DNA Ligase and PARP inhibitors are therapeutic targets in TKI resistant chronic myeloid leukemia

Lisa Tobin, Annahita Sallmyr, Aaron Rapoport, Alan E. Tomkinson,
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The BCR-ABL fusion gene in Philadelphia (Ph)-positive CML encodes a constitutively active tyrosine kinase that leads to increased ROS, repair errors and genomic instability. We have previously shown that an error-prone alternative NHEJ pathway involving DNA ligase III α /XRCC1 is upregulated in BCR-ABL positive CML, providing a mechanism for generating repair errors and genomic instability. "Knockdown" of these proteins in CML cells leads to decreased DNA repair and an increased frequency DSBs, indicating that this pathway is important to the survival of BCR-ABL-positive cells, and so may be a specific therapeutic target. We have recently identified inhibitors of the human DNA ligases and have shown that these molecules potentiate the cytotoxic effects of DNA damaging agents in cancer cells. Because PARP-1 inhibitors have been shown to specifically kill cells with defects in DSB repair, and BCR-ABL-positive cells have abnormal DSB repair, we have examined the effect of PARP-1 inhibitors on BCR-ABL-+ve cells in the presence or absence of DNA ligase inhibitors. We find that ligase inhibitors in combination with PARP inhibitors lead to a significant decrease in colony survival of imatinib resistant (IR) BCR-ABL positive cells (IRP210Mo7e), compared with BCR-ABL cells that are imatinib sensitive (P210Mo7e) and control cells (Mo7e). Importantly, a subset of IR CML patient samples (N=5) treated with these inhibitors

show a decrease in colony survival, compared with those samples that are imatinib sensitive. In addition Baf3 cells stably expressing BCR-ABL mutations that confer IR, such as T315I and E255K respond differently to the ligase and PARP inhibitor combinations: E255K is sensitive whereas T315I is not. While both CML sensitive and resistant cells show similar increases in DNA damage following drug treatment, It appears that DNA repair is altered in IR cells. We therefore suggest that while CML cell survival is at least in part maintained by repair of DSBs using "back-up" NHEJ, IR cells have alterations in this and/or other DSB repair pathways. Studies to identify the mechanism(s) by which imatinib resistance affects DSB repair pathways are underway.

International Symposium on "RECENT PROGRESS IN CANCER THERAPEUTICS"

November 12-13, 2008

at Chulabhorn Convention Center, Bangkok, Thailand

**INHIBITING DOUBLE STRAND BREAK REPAIR AND ALTERNATIVE NON HOMOLOGOUS
ENDJOINING: POTENTIAL THERAPEUTIC TARGETS IN CHRONIC MYELOID LEUKEMIA (CML)
WITH RESISTANCE TO IMATINIB?**

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Department of Radiation Oncology, University of Maryland, School of Medicine, Baltimore, Maryland, USA1.

BCR-ABL fusion tyrosine kinase in chronic myeloid leukemia (CML), induces high levels of ROS that generate DNA double strand breaks (DSBs). We previously showed that CML cells repair DSBs by aberrant non homologous end-joining (NHEJ) that is characterized by large DNA deletions. The generation of DNA alterations represents a mechanism by which genomic changes may be acquired in the progression of chronic phase CML to blast crisis. Recently, we demonstrated that a "back-up" or alternative NHEJ pathway is involved in aberrant repair of DSBs in CML. Proteins in this pathway include, DNA ligase III α , XRCC1 and poly(-ADP) ribose polymerase (PARP). In particular, we have demonstrated that alternative NHEJ proteins, such as DNA ligase III α are over-expressed in CML. This increased expression appears to be dependent on the presence of BCR-ABL. "Knockdown" of these proteins leads to an accumulation of unrepaired DSBs, demonstrating their essential involvement in DSB repair in CML cells.

Imatinib that targets BCR-ABL is the gold standard for therapy in CML patients. However, it has become clear that patients become resistant to imatinib and other tyrosine kinases, such as dasatinib and nilotinib. This suggests an urgent need for alternative therapeutic targets for patients resistant to tyrosine kinase inhibitors. The recent development of a new generation of PARP inhibitors and our recent identification of DNA Ligase inhibitors by computer aided drug design (CADD) has prompted us to test BCR-ABL-positive and isogenic imatinib resistant cell lines in DNA damage, proliferation

and colony survival assays. Small molecule inhibitors of DNA ligase I/III combined with PARP inhibitors result in a significant increase in DNA double strand breaks in both BCR-ABL-positive and imatinib resistant cells. Interestingly, colony survival is significantly decreased in imatinib resistant cells, compared with non-imatinib resistant controls. Our data suggest that while CML cell survival is at least in part maintained by repair of DSBs using "Back-up" NHEJ, imatinib resistant cells may be more primed to repair of DSB using this alternative pathway. Thus, inhibition of this pathway may have a greater effect on cell survival. In conclusion, the main proteins involved in alternative NHEJ, which include DNA ligase III α , XRCCI, DNA Ligase I, PARP and WRN have the potential to be novel therapeutic targets in CML patients that have acquired resistance to imatinib.

2. Invited Talks

International Symposium on

“RECENT PROGRESS IN CANCER THERAPEUTICS”

November 12-13, 2008

at Chulabhorn Convention Center, Bangkok, Thailand

Co-Chairpersons:- Prof. Dr. HRH Princess Chulabhorn Mahidol (*Chulabhorn Research Institute, Thailand*)

- Enrico Mihich (*Roswell Park Cancer Institute, U.S.A.*)

- Kurt S. Zänker (*Institute of Immunology, University of Witten/Herdecke, Germany*)

Secretary General:- Khunying Mathuros Ruchirawat (*Chulabhorn Research Institute, Thailand*)

November 12, 2008

08:00 - 09:15 REGISTRATION

09:30 OPENING CEREMONY:

- Report by *Khunying Mathuros Ruchirawat*

- Focus and Goals by *Dr. Enrico Mihich*

- Opening Address of *Prof. Dr. HRH Princess Chulabhorn Mahidol*

- Opening Keynote Lecture: Genetic Alterations in Nasopharyngeal Carcinoma in the Thai Population by *Prof. Dr. HRH Princess Chulabhorn Mahidol*

10:15 Break

Session I: Molecular Targets for Potential Exploiment

Chairpersons: - Frank Entschladen (*University of Witten/Herdecke, Germany*)

- M.R. Jisnusun Svasti (*Chulabhorn Research Institute, Thailand*)

10:30 The cancer epigenome---Origins biological and translational implications

Stephen B. Baylin (*Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, U.S.A.*)

11:15 The role of *Thoc1* in prostate cancer

David W. Goodrich (*Roswell Park Cancer Institute, U.S.A.*)

12:00 Cadmium carcinogenesis: The Wnt-beta catenin signaling pathway as a novel target of therapy?

Frank Thévenod (*University of Witten/Herdecke, Germany*)

12:45 LUNCH

Date: 7-Nov-08

Session II: Molecular Target-Oriented Therapeutics

Chairpersons: - Soldano Ferrone (*University of Pittsburgh Cancer Institute, U.S.A.*)

- Khunying Mathuros Ruchirawat (*Chulabhorn Research Institute, Thailand*)

14:00 The future of epigenetic therapy

Peter A. Jones (*USC/Norris Comprehensive Cancer Center, U.S.A.*)

14:45 Overexpression of some DNA repair pathways are associated with metastasis risk in melanoma patients

Alain Sarasin (*Institute Gustave Roussy, France*)

15:30 Inhibiting double strand break repair and alternative non homologous endjoining: Potential therapeutic targets in chronic myeloid leukemia (CML) with resistance to imatinib?

Feyruz Rassool (*University of Maryland, U.S.A.*)

November 13, 2008

Session III: Therapeutics Modification of Tumor Microenvironment

Chairpersons: - Alberto Mantovani (Istituto Clinico Humanitas, Italy)

- Skorn Mongkolsuk (Chulabhorn Research Institute, Thailand)

09:30 **Keynote Address:** Mechanisms of malignant progression

Robert A. Weinberg (*Whitehead Institute for Biomedical Research, U.S.A.*)

10:15 TGF-beta regulation of the tumor microenvironment

Li Yang (*Vanderbilt-Ingram Cancer Center, U.S.A.*)

11:00 Break

11:30 Neoneurogenesis: Mechanisms of tumor innervation and its consequences for metastasis formation

Frank Entschladen (*University of Witten/Herdecke, Germany*)

12:15 Aminoacid deprivation, Autophagy, Apoptosis (AAA): Targeted therapy in cancer

Niramol Savaraj (*University of Miami, U.S.A.*)

13:00 LUNCH

Date: 7-Nov-08

Session IV: Translational Investigations

Chairpersons: - Frank Thévenod (University of Witten/Herdecke, Germany)

- Kavi Ratanabanangkoon (Chulabhorn Research Institute, Thailand)

14:00 Inflammation and cancer: The dark side of the force

Alberto Mantovani (*Istituto Clinico Humanitas, Italy*)

14:45 Antitumor effects mediated by targeting of tumor-associated activated pericytes

Soldano Ferrone (*University of Pittsburgh Cancer Institute, U.S.A.*)

15:30 Break

16:00 Dissecting RNA mediated gene silencing pathways in murine embryonic stem cells

Michael Rossbach (*Genome Institute of Singapore, Singapore*)

16:45 **Concluding Remarks**

Kurt S. Zänker (*Institute of Immunology, University of Witten/Herdecke, Germany*)

3. Published Manuscripts

Up-regulation of WRN and DNA ligase IIIalpha in chronic myeloid leukemia: consequences for the repair of DNA double-strand breaks.

Sallmyr A, Tomkinson AE, Rassool FV.

Blood. 2008 Aug 15;112(4):1413-23. Epub 2008 Jun 4.

Genomic instability in myeloid malignancies: increased reactive oxygen species (ROS), DNA double strand breaks (DSBs) and error-prone repair.

Sallmyr A, Fan J, Rassool FV.

Cancer Lett. 2008 Oct 18;270(1):1-9. Epub 2008 May 7. Review.